How do anti-mitotic drugs kill cancer cells?

Karen E. Gascoigne* and Stephen S. Taylor‡

Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK

*Present address: The Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02142, USA

‡Author for correspondence (e-mail: stephen.taylor@manchester.ac.uk)

Journal of Cell Science 122, 2579-2585 Published by The Company of Biologists 2009
doi:10.1242/jcs.039719

Summary

In 2007, over 12-million people were diagnosed with cancer. According to the American Cancer Society, at least one third of these individuals are not expected to survive the disease, making cancer the second most prevalent cause of death worldwide. Systemic chemotherapy forms the mainstay of cancer treatment, and agents that disrupt mitotic spindle assembly – so called 'anti-mitotics' – are commonly used to treat a wide variety of cancers. Traditional anti-mitotic agents include the microtubule toxins such as taxol, other taxanes and the vinca alkaloids, all of which have proven successful in the clinic. However, patient response remains highly unpredictable, and drug resistance is common. In addition, toxicity is a problem. To address these limitations, a new generation of anti-mitotic drugs is being developed. As the first wave of these new agents enters clinical trails, much hope rests on their outcome. Meanwhile, significant attention is being focused on trying to predict which tumour types are likely to respond. In this Commentary, we outline recent advances in our understanding of how cancer cells respond to anti-mitotic drugs, and discuss the relevance of these studies to their use in the clinic.

Key words: Taxol, Paclitaxel, Spindle checkpoint

Introduction

Drugs that disrupt mitotic progression, which are commonly referred to as 'anti-mitotics', are used extensively for the treatment of cancer. Currently, all such drugs that have been approved for clinical use target microtubules, with the taxanes and vinca alkaloids showing much success against a number of cancers. Taxol is commonly used in the treatment of breast and ovarian cancers, and vinca alkaloids, such as vincristine, are often used in combination therapies to treat haematological malignancies (reviewed by Jordan and Wilson, 2004). Investigating the effects of these agents on microtubule dynamics (Box 1) has revealed much about their mechanism of action. The vinca alkaloids, which are a class of compounds that were originally isolated from *Catharanthus roseus* (madagascar periwinkle), interact with β-tubulin at a region adjacent to the GTP-binding site known as the vinca domain (Rai and Wolff, 1996). Within a concentration range that blocks proliferation, the vinca alkaloids bind to tubulin at the plus-tip of microtubules. At the lower end of this range, this inhibits microtubule dynamics without altering polymer levels, whereas at higher concentrations, it induces microtubule depolymerisation (Jordan et al., 1991). In both situations, mitotic spindle formation is disrupted, and cells therefore fail to complete a normal mitosis (Jordan et al., 1991). At very high concentrations (above 10 μM) vinca alkaloids can induce the aggregation of tubulin into para-crystals; however, this does not occur at clinically relevant concentrations (Jordan and Wilson, 1999).

Taxol, which was originally derived from the bark of *Taxus brevifolia* (pacific yew tree), stabilises microtubules and dampens the dynamics of the polymer, thereby reducing depolymerisation (Schiff et al., 1979). In mammalian cells, low concentrations of taxol stabilise microtubules, whereas higher concentrations increase polymerisation (Jordan et al., 1993). Taxanes bind β-tubulin, but only when the monomer is incorporated into a microtubule. The binding site for taxol is on the inner face of the polymer, and the drug can bind the length of the polymer. Drug binding is thought to stabilise the structure of the polymer by inducing a conformational change, which enhances the affinity of the interaction between tubulin molecules (Nogales, 2000). Stabilisation of microtubules by taxol binding prevents normal formation of mitotic spindles (Jordan et al., 1996). On entry into mitosis, chromosomes can attach taxol-stabilised microtubules; however, the lack of microtubule dynamics means that tension is not produced across sister chromatids (Kelling et al., 2003), and prevents correct chromosome bi-orientation. This leads to chronic activation of the spindle-assembly checkpoint (SAC), which in turn leads to mitotic arrest (Musacchio and Salmon, 2007).

Although the mechanisms by which anti-mitotic drugs elicit a mitotic arrest are now well understood, relatively little is known about how cells respond to this prolonged cell-cycle delay. Recently, however, several studies have taken a new approach, using high-content imaging and live-cell analysis to monitor the long-term behaviour of cells in response to anti-mitotic drugs. In this Commentary, we focus on these recent studies and how they have advanced our understanding of how cancer cells respond to anti-mitotic drugs, at least in cell culture. We also discuss the relevance of these new findings to the clinical use of both classical and novel anti-mitotic agents.

The clinical use of anti-mitotics

Although microtubule toxins have shown great success in the clinic, two factors – namely resistance and toxicity – have limited their effectiveness. Patient resistance to classic anti-mitotic agents, particularly to taxol, is commonly observed (McGrogan et al., 2008). Some patients respond well to treatment, but others rapidly acquire resistance and show little improvement. Toxicity is also a major limitation: in addition to killing tumour cells, anti-mitotic agents affect the division of normal cells, which therefore manifests as myelosuppression. Furthermore, because these agents also disrupt microtubule dynamics in non-dividing cells such as peripheral neurons, neuropathies can also develop. Myelosuppression is reversible and therefore clinically manageable. By contrast, neurotoxicities are more problematic because they can often cause permanent damage (Rowinsky et al., 1993).

To minimise neurological side effects, new agents are being developed that disrupt mitosis without interfering with microtubule

Box 1. Microtubule dynamics

Microtubules are made of α- or β-tubulin heterodimers that polymerise to create protofilaments, 13 of which then bundle to form a hollow cylinder. These tubes can grow and shrink by the addition or removal of subunits from the ends. Because microtubules are made up of heterodimers, they are polarised, with α-tubulin exposed at the slow-growing minus end. The plus end terminates with β-tubulin, where the addition and removal of subunits is faster. In cells, microtubules are nucleated from the minus end, which is typically embedded into the centrosome, whereas the plus end grows out into the cell. Both the α- and β-subunits bind GTP, and the β-subunit hydrolyses the GTP once it is incorporated into a microtubule. During elongation of the microtubule, GTP hydrolysis lags behind subunit addition, thus forming a 'GTP cap' at the plus end. This stabilises the plus end, promoting further extension of the polymer. However, when the rate of subunit addition decreases, hydrolysis 'catches up' and the GTP cap is lost, promoting rapid depolymerisation of the microtubule. The switch from growth to shrinkage is known as 'catastrophe', and the switch from shrinkage to growth is known as 'rescue'. This phenomenon whereby microtubules can grow, shrink and rapidly switch between these two states is known as 'dynamic instability'. Importantly, this special property of microtubules means that, within a population of microtubules, some microtubules can be growing while others are shrinking. Dynamic instability is especially important during mitosis, because it allows rapid assembly of the mitotic spindle. In addition, as microtubules grow and shrink, they efficiently probe the three-dimensional space around the spindle, facilitating chromosome capture and alignment. Agents that interfere with microtubule dynamics profoundly disrupt mitosis by preventing normal spindle assembly and chromosome segregation. For a comprehensive review on how microtubule toxins interact with microtubules, see Jordan and Wilson (Jordan and Wilson, 2004).

dynamics in non-dividing cells. The rationale of this approach is that such drugs should prevent assembly of the mitotic spindles, and thereby retain anti-tumour activity, but not induce neuropathies. Frontrunners in this new class of therapeutics are inhibitors of the Eg5 kinesin, a motor protein that is required for the separation of spindle poles during mitosis. Agents are also being developed that inhibit mitotic kinases such as Aurora A and polo-like kinase 1 (Plk1), both of which also play a role in spindle formation (reviewed by Bergnes et al., 2005; Keen and Taylor, 2004; Strebhardt and Ullrich, 2006). One of the most advanced new agents is the Eg5 inhibitor Ispinesib, which has entered phase II trials for metastatic melanoma, hepatocellular carcinoma and cancers of the head and neck (Knox et al., 2008; Lee et al., 2008; Tang et al., 2008). Encouragingly, minimal neurotoxicity has been observed. However, the key question now is whether Ispinesib, or any of the other new anti-mitotic agents, will have clinical efficacy. To achieve this, it will be important to identify which tumours are most likely to respond to these agents. In turn, this requires an understanding of the basic mechanisms by which anti-mitotic agents kill tumour cells.

Cell culture as a model system for studying anti-mitotics

Despite extensive research, the mechanism by which anti-mitotic drugs cause cell death is still poorly understood (Rieder and Maiato, 2004). Much of our understanding of the action of anti-mitotic drugs is based on work carried out using cell culture systems. What is clear from these studies is that disruption of the mitotic spindle activates the SAC, thereby inducing a prolonged mitotic arrest that ultimately leads to cell death (Rieder and Maiato, 2004). However, the molecular links between these two events are far from clear. Furthermore, there is considerable ambiguity regarding when during the cell cycle drug-treated cells die. Some studies show that, following drug exposure, cells exit mitosis without undergoing cell division and remain in a tetraploid state (Jordan et al., 1996; Tao et al., 2005) (Fig. 1). In this case, some studies report that cells die in the subsequent interphase, or following one or more additional cell cycles (Jordan et al., 1996). Other studies indicate that cell death occurs directly during mitosis (Panvichian et al., 1998), whereas others report that cells undergo an abnormal division (Chen and Horwitz, 2002). Therefore, the timing of cell death in relation to progression through the cell cycle is unclear. Similarly, the mechanism of cell death is equally uncertain: whereas some studies suggest that a classical apoptotic response occurs (Allan and Clarke, 2007; Panvichian et al., 1998), others suggest a form of caspase-independent death (Niikura et al., 2007).

The fact that multiple outcomes have been described following exposure to anti-mitotic agents raises two possibilities. First, the variation could be due to technical issues; indeed, the vast differences in the experimental strategies used (for example, choice of cell line, drug, drug concentration, type of analysis) makes drawing direct comparisons between the results of different studies impossible. Alternatively, the different outcomes could indicate that cells can elicit different responses to drug treatment. In turn, this suggests that the observed variation in clinical responses might be reflected in the cell culture models. Importantly, if this is the case, then the identification of the molecular determinants that underlie this variation might allow clinicians to better predict patient responses to a given drug. Indeed, much effort has been directed at trying to decipher the molecular determinants of cell fate in response to prolonged mitotic arrest (Rieder and Maiato, 2004). Unfortunately, careful interpretation of the data derived from the majority of studies in this area is difficult because they use either cell-population-based methods (such as flow cytometry or immunoblotting), or rely on the analysis of fixed cells (for example, immunofluorescence). None of these approaches yield information regarding the 'history' of any given cell or the kinetics of its response to drug treatment. Simply put, because such analyses cannot tell whether an apoptotic cell executed cell death in mitosis or interphase, the link between mitosis and cell death cannot be assessed.

Recent developments: single-cell assays show the way

Recently, several studies have started to tackle the limitations that have hampered progress thus far. Technological advances with live-cell imaging have allowed researchers to carry out more detailed studies that describe the behaviour of single cells in response to anti-mitotic drugs (Brito and Rieder, 2008; Brito et al., 2008; Gascoigne and Taylor, 2008; Orth et al., 2008; Shi et al., 2008). By using a high-content screening approach, Shi et al. compared the response of several cancer cell lines to anti-mitotic drug treatment, and noted that the number of cells that died in response to drug treatment varied widely between cell lines (Shi et al., 2008). By using immunoblotting, they found that the sensitivity of the cells to anti-mitotic drugs correlated with the loss of the expression of X-linked inhibitor of apoptosis (XIAP) during the drug response. Work from the same group investigating the response of cells to a novel Eg5 inhibitor identified similar variation in response of the different cell lines (Orth et al., 2008). Orth and colleagues used a

bright-field time-lapse imaging approach to monitor the response of cells to drug treatment. Significantly, the authors observed that the drugs induced greater cell death in cancer cell lines than in non-transformed cells, and noted particularly rapid cell killing in a leukemia cell line. In a similar study, time-lapse analysis of non-transformed retinal pigment epithelial (RPE) cells that had been exposed to a variety of anti-mitotics indicated that the duration of mitotic arrest in these cells was highly dependent on the anti-mitotic drug used (Brito et al., 2008). Importantly, Brito and colleagues showed that the duration of mitotic arrest was not determined by the presence or absence of microtubules. These authors used the same approach to compare the response of RPE cells to two cancer cell lines, HeLa and U2OS (Brito and Rieder, 2008), to different anti-mitotic drugs. They also observed a wide variation in the response, both in the duration of mitotic arrest and the amount of cell death, and noted greater cell death in cancer cells. Intriguingly, this study showed that cell death in RPE and HeLa cells – but not in U2OS cells – was caspase-dependent, which suggests that different cell death mechanisms might be induced by anti-mitotic drugs in different cell types. The authors also noted that, in addition to inhibiting cell death, the presence of a pan-caspase inhibitor extended the duration of mitotic arrest in RPE cells.

Work from our laboratory revealed a similar phenomenon (Gascoigne and Taylor, 2008). By carrying out time-lapse imaging of 13 cancer cell lines and two non-transformed cell lines (all of which expressed a GFP-tagged histone to monitor chromatin morphology), we analysed cell behaviour in response to a number of anti-mitotic drugs. We uncovered wide variation in the response of cells, both within many cell lines (intraline variation) and between cell lines (interline variation). Cell fate was found to depend on the anti-mitotic drug used and on its concentration, but not on the duration of mitotic arrest. Cell death was found to be caspase-dependent and could be inhibited by a pan-caspase inhibitor, which also extended the duration of mitotic arrest.

A new level of complexity

When considered together, a striking consensus emerges from these studies. All of them show an unprecedented level of variation in the response to anti-mitotic drugs and, importantly, have enabled a number of pre-existing hypotheses to be ruled out. All of the above studies noted that the particular anti-mitotic drug used, as well as the concentration, could dramatically affect the response of cells. However, there was very little correlation between the duration of mitotic arrest and cell death. This calls into question the long-held dogma that the longer a cell spends in mitosis, the more likely it is to die, and that the strength of SAC signalling somehow plays a role in cell fate (for reviews, see Rieder and Maiato, 2004; Weaver and Cleveland, 2005). Similarly, several of these studies observed that cell death could occur during mitosis and/or after mitotic exit, indicating that mitotic exit is not required for these agents to elicit cell killing, as has previously been suggested (Tao et al., 2005).

In the above studies, variation was noted not just between cell lines but also within individual populations of genetically identical cells. Therefore, the response of a cell line should be considered more as a ‘profile’ of fates, rather than a single response (Fig. 2). Although uncovering the complexity of the response to anti-mitotic drugs does in itself represent a major breakthrough, the key question remains: what are the molecular mechanisms that are responsible for these different behaviours? Recent work has started to address this question and, in particular, the factors that control whether an individual cell exits mitosis or dies in mitosis have recently started to be uncovered.

During an unperturbed mitosis, mitotic exit is triggered by the rapid degradation of cyclin B1. Cyclin B1 degradation is prevented

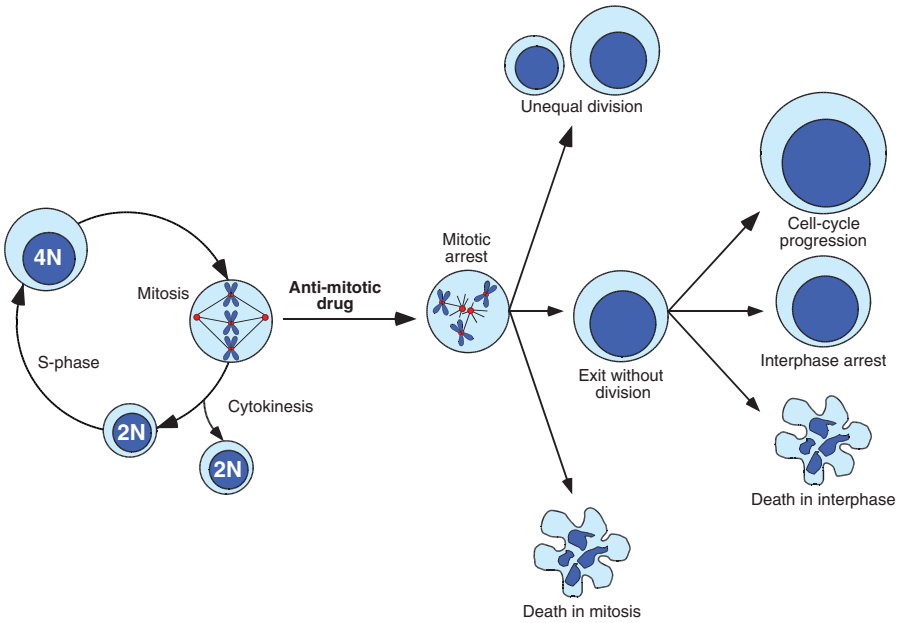

Fig. 1. Cell fate in response to anti-mitotic drug treatment. When cells are exposed to an anti-mitotic agent such as taxol, they arrest in mitosis due to chronic activation of the spindle-assembly checkpoint. They then undergo one of several fates. Cells might die directly in mitosis, or divide unequally to produce aneuploid daughter cells. Alternatively, cells might exit mitosis without undergoing division. In this case, cells might then die in interphase, arrest in interphase indefinitely or enter additional cell cycles in the absence of division.

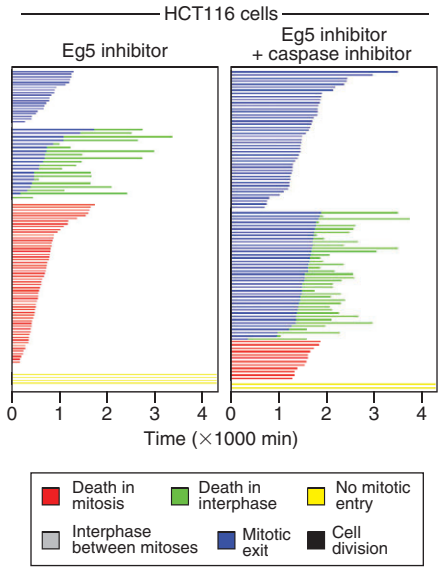

Fig. 2. The response of cells to anti-mitotic drugs is highly complex. HCT116 colon cells expressing GFP-histone H2B were followed by time-lapse imaging for 72 hours in the presence of an Eg5 inhibitor (1 μM AZ138) with or without a pan-caspase inhibitor (100 μM Boc-D-FMK). The duration of mitosis and the fate of the cell were recorded for 100 cells in each condition. On the fate profile, each horizontal bar represents one cell, the colour of the bar denotes the fate of the cell and the length of the bar denotes the duration of that fate. Note that caspase inhibition reduces the number of cells that die directly from mitosis (red bars) and prolongs the duration of mitosis (length of bars). Data are reproduced with permission from (Gascoigne and Taylor, 2008).

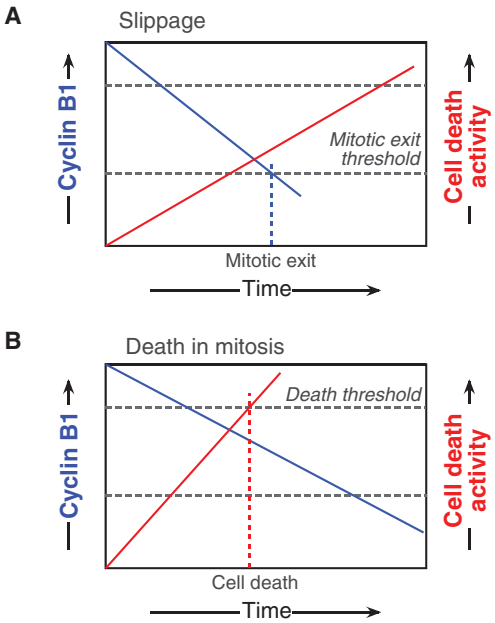

Fig. 3. The competing-networks model. The decision to die in mitosis or to exit and return to interphase (slippage) is dictated by two competing networks, one that involves the activation of cell death pathways and the other that protects cyclin B1 from degradation. During a prolonged mitotic arrest, cell death signals become stronger while cyclin B1 levels fall. Both networks have thresholds and the fate of the cell is dictated by which threshold is breached first. Thus, if cyclin B1 levels fall below the mitotic-exit threshold first, slippage occurs (A). If the death threshold is breached first, then the cell dies in mitosis (B). Model reproduced with permission from (Gascoigne and Taylor, 2008).

by the activity of the SAC until chromosomes are correctly aligned. It now appears that, in mammalian cells exposed to anti-mitotic agents, cyclin B1 is still slowly degraded despite chronic activation of the SAC (Brito and Rieder, 2006; Gascoigne and Taylor, 2008). Eventually, the amount of cyclin B1 falls below the threshold that is required to maintain the mitotic state and the cell exits mitosis, a process known as mitotic 'slippage' (Brito and Rieder, 2006). Consistently, when cyclin B1 is overexpressed, it takes longer for the exit threshold to be breached and thus mitotic exit is delayed (Gascoigne and Taylor, 2008). Importantly, the rates of slippage differ both within a population and between populations, and correlate with cell fate in many instances. Therefore, it is clear that defining the factors that govern the rate at which cyclin B1 is slowly degraded during a prolonged mitotic arrest is an important goal for future work.

Understanding mitotic cell death

With respect to the mechanism that underlies mitotic cell death in response to anti-mitotic drugs, several recent studies have identified the process as being caspase-dependent, as a variety of pan-caspase inhibitors prevented or delayed cell death (Brito and Rieder, 2008; Gascoigne and Taylor, 2008; Shi et al., 2008) (Fig. 2). Moreover, caspase inhibition was frequently found to prolong the duration of mitosis. A trivial explanation for this is that caspase activity is required for mitotic slippage. However, measuring cyclin B1 degradation in the presence of a caspase inhibitor indicates that this is not the case (K.E.G. and S.S.T., unpublished). To explain the ability of the caspase inhibitor to prolong mitosis, we recently proposed a new model whereby cell fate is determined by two competing networks, one that involves the activation of cell death pathways, and another that controls the degradation of cyclin B1 and thus exit from mitosis (Fig. 3). We propose that these two networks work in opposite directions during a mitotic arrest: while cell death signals become stronger, cyclin B1 levels fall. Both networks have thresholds, and whether a cell undergoes death is determined by a 'race' between the two networks – whichever threshold is breached first determines the fate. Thus, if cyclin B1 levels fall below the mitotic-exit threshold first, slippage occurs. If the death threshold is breached first, the cell dies in mitosis. Consistent with this model, one study noted that anti-mitotic drug sensitivity correlated with the levels of the inhibitor of apoptosis protein XIAP (Shi et al., 2008). This suggests that cell lines in which XIAP is degraded more rapidly might activate cell death networks more quickly than others. Clearly, another important goal for future work is to define the factors that govern the rate at which death signals accumulate during a prolonged mitotic arrest.

Although our knowledge of the cell death pathways at play here is rudimentary, it is reasonable to speculate that the level of cyclin-dependent kinase 1 (Cdk1) activity could be crucial to the control of both cell death and mitotic exit. A recent study showed that Cdk1-mediated phosphorylation of caspase-9 at Thr125 reduces its activity (Allan and Clarke, 2007). When this residue was mutated to prevent the phosphorylation of caspase-9, cell death in response to anti-mitotic drugs was greatly increased. This leads to the intriguing hypothesis that levels of cyclin B1 – and therefore the activity of Cdk1 – could control both mitotic exit (via slippage) and cell death (via the inhibition of the apoptotic machinery). In this case, the commitment to either fate would then be determined by the relative requirement for Cdk1

activity in the two networks, and in which network the 'Cdk1-activity threshold' was breached first.

The post-mitotic response

Although the 'competing-networks' model described above (Fig. 3) provides a useful framework by which to describe the decision of a cell to either die during mitosis or exit mitosis, it does not easily explain the variety of behaviors that are observed following mitotic exit – the post-mitotic response. Several fates have been described for cells that exit mitosis in the presence of an anti-mitotic drug, including cell-cycle arrest, apoptosis and cell-cycle progression (Fig. 1). The molecular factors that govern these fates are not well understood, but p53 appears to be involved. Substantial evidence supports the theory that p53 restrains cell-cycle progression following exit from a prolonged mitotic arrest (Lanni and Jacks, 1998). It is unclear whether a p53-dependent response is induced during mitosis, or by a de novo signal that arises after mitotic exit. One possibility is that damage or stress that has accumulated during mitosis does not always trigger an apoptotic response during mitosis due to the inhibitory action of Cdk1 on caspase-9 (Allan and Clarke, 2007). Following mitotic exit, and the loss of this Cdk1-mediated inhibition, the apoptotic threshold might fall to its interphase set-point. In turn, the pre-existing damage signal is then recognised, leading to execution of the apoptotic program. Thus, it is likely that the fate of the cell in response to drug treatment is determined not only by events occurring during a mitotic arrest, but also the by the consequences of these events after mitotic exit, as well as additional signaling pathways that are active during interphase.

The clinical significance of cell culture experiments

Although the results of recent cell culture studies have been highly informative, they will not be of great use if they bear no relation to events in an in vivo context. Significantly, a pair of studies that investigated the effect of taxanes on several mouse tumour models also observed wide variation in the response. Tumour regression that was induced by taxane treatment varied widely, and the mitotic index of cells within the tumour did not correlate with either cell death or tumour regression (Milross et al., 1996; Schimming et al., 1999). Similar observations were made in a highly informative clinical study. Eleven women that received neoadjuvant taxol treatment for non-metastatic breast cancer were followed and, at regular intervals post-treatment, biopsies and mammograms were used to determine the mitotic index, apoptotic index and tumour size (Symmans et al., 2000). The authors observed a wide variation in both the mitotic and apoptotic index in response to the drug, with no correlation observed between the two measures. Therefore, the complexity that has been unveiled by recent cell culture studies might indeed reflect the complex patient responses that are observed in the clinic.

An important factor that must be acknowledged when considering the relevance of cell culture data is the availability of drug in vivo. In the cell culture studies discussed here, cells were continually exposed to a constant concentration of anti-mitotic agent. In reality, the concentration of drug experienced by a tumour in vivo will vary. The majority of anti-mitotic agents are administered intravenously, and therapy typically involves treatment cycles of only a few hours, on a weekly basis. Thus, drug concentrations increase, peak and fall as the compound is circulated and then removed from the body. For example, the elimination half-life for taxol is around 20 hours and, for three-hour treatments, maximal plasma concentrations typically occur within the first five hours (Karlsson et al., 1999; Rowinsky, 1997). Thus, exposure of the tumour to an effective dose is likely to be transient. Cell culture experiments have identified drug concentration as a crucial factor in the response to anti-mitotics. Therefore, it is likely that the varying concentration of drug experienced by cancer cells in vivo will add an additional layer of complexity to the response of the tumour as a whole.

Although the phase II trials with the Eg5 inhibitor Ispinesib caused minimal neurotoxicity, these studies failed to show substantial clinical benefit in the cancers tested (Knox et al., 2008; Lee et al., 2008; Tang et al., 2008). Previous preclinical studies and more recent live-cell imaging experiments do not suggest that Eg5 inhibitors will be any less effective than current anti-mitotic drugs in terms of cytotoxicity. Therefore, data from cell culture models might not be directly applicable to the in vivo situation in this case. However, the lack of significant responses in the Ispinesib trials might simply be because the wrong tumour types were tested; cancers of the breast and ovary, in which taxol is particularly effective, have not yet been tested in clinical trials.

The new anti-mitotic drugs are still in the early stages of clinical development, but it might nevertheless be prudent to imagine a 'worst-case scenario' and ask why these drugs have not yet shown promising results in the clinic. In this regard, it is worth considering the reason for the clinical efficacy of the microtubule toxins. It is possible that the clinical efficacy of taxol is not in fact due to its disruption of mitosis, but rather to an 'off-target' effect. For example, a possibility that has not yet been fully explored is whether drugs such as the taxanes affect tumour vasculature by disrupting the function and/or proliferation of new blood vessels that are required to provide nutrients to the growing tumour (Schwartz, 2009). If this is the case, microtubule toxins might in effect mediate a 'double-whammy' by directly inhibiting tumour cell proliferation and indirectly inhibiting tumour growth by restricting angiogenesis.

Another possible explanation for why taxol is particularly effective could be related to a little-noted concentration-dependent effect that has been observed in cell culture. Most anti-mitotic drugs, including Eg5 inhibitors, have an all-or-nothing effect on cell division; they have no observable effect at low drug concentrations but induce a robust mitotic arrest when administered above a crucial concentration (Chen and Horwitz, 2002; Gascoigne and Taylor, 2008; Klein et al., 2007). However, in response to taxol, abnormal DNA contents and cell death were reported at low drug concentrations, where a robust mitotic arrest was not apparent (Chen and Horwitz, 2002). This suggests that anti-mitotic agents that elicit their effect via prolonged mitotic arrest are only cytotoxic at a high concentration. By contrast, taxol might be capable of preventing proliferation at lower concentrations, such as those that are present in vivo as the compound starts to be cleared from the body.

Tumour-specific therapies

The clinical success of the microtubule toxins suggests that mitotic disruption is an effective anti-cancer strategy. However, although the development of anti-mitotic drugs that do not disrupt microtubule dynamics appears to have circumvented the problem of neurotoxicity, the issue of immunotoxicity remains unresolved. Preliminary data from the Ispinesib trials indicate that significant neutropenia is induced by the drug, which suggests that the use of this agent will be restricted in a similar manner to the currently used compounds (Tang et al., 2008). As an alternative to anti-mitotic drugs, the mitotic kinase Aurora B is also being explored as an anti-cancer drug target. Rather than preventing mitotic progression, drugs that inhibit Aurora B cause premature mitotic exit in the presence

of unaligned chromosomes and a failure of cytokinesis, which ultimately leads to cell death (Keen and Taylor, 2004). As a 'mitotic driver' rather than an inhibitor of mitotic progression, Aurora B inhibitors offer an alternative strategy to target dividing cells (Keen and Taylor, 2009). However, Aurora B inhibitors are still cytotoxic agents that kill normal dividing cells in addition to cancer cells and, as such, they are expected to induce the myelosuppression that is typical of the traditional anti-mitotic agents.

To address these problems, the search has intensified for more 'tumour-specific' therapies. Much current research is focused on identifying situations in which tumour cells are dependent on particular pathways or proteins that are non-essential in normal cells. It has long been known that many cancer cells contain abnormal numbers of centrosomes. However, cancer cells commonly cluster these centrosomes during mitosis and thereby avoid a potentially lethal multi-polar mitosis (Brinkley, 2001). A recent RNA interference screen was designed to identify proteins that are required for centrosome clustering and revealed that the minus-end-directed microtubule motor protein HSET (also known as KIFC1, kinesin family member C1) is involved in this process (Kwon et al., 2008). Strikingly, the depletion of HSET inhibited the proliferation of cancer cells with extra centrosomes but had little effect on normal cells with only two centrosomes. This observation suggests that it might indeed be possible to exploit the mitotic machinery to develop cancer-specific therapies.

The competing-networks model also offers the potential for more tumour-specific targeting (Gascoigne and Taylor, 2008). The model predicts that, in cells in which there is a fine balance between the pathways that control slippage and cell death, apoptosis-promoting strategies could be particularly effective. Several studies have recently noted that non-transformed RPE cells undergo much less cell death in response to anti-mitotic drug treatment than many cancer cell lines (Brito and Rieder, 2008; Gascoigne and Taylor, 2008; Orth et al., 2008). This implies that cancer cells might be closer to this cell fate 'decision point' than normal dividing cells during a drug-induced mitotic arrest, and that cancer cells might therefore be more sensitive to apoptosis-promoting strategies. Consequently, a therapy that combines an anti-mitotic agent with an apoptosis-inducer might induce a more potent anti-tumour effect (for discussion, see Holland and Cleveland, 2008).

Conclusions and perspectives

Predicting the response of patients to anti-mitotic agents remains a challenge in the clinical setting, but the recent cell culture studies discussed here have started to shed some light on this variable response. Such studies have shown that individual cancer cells can exhibit widely varying responses to anti-mitotic drugs, which might be in part due to the competing action of the pathways that control cell death and mitotic slippage. Although this is an important advance in our understanding of why responses to these drugs are variable, many questions remain unanswered. From a clinical point of view, obtaining a better understanding of the apoptotic trigger for mitotic cell death, and how this varies between cells, is an important goal. For example, why do some cancer cells take twice as long as others to execute cell death in response to taxol treatment (Gascoigne and Taylor, 2008)? Is the mechanism that controls this difference also responsible for the varying apoptotic index observed in tumour samples taken from patients following taxol treatment (Symmans et al., 2000)?

This is a crucial time for the field of anti-mitotic drug research. As we eagerly await the results of clinical trials that will test the next generation of anti-mitotic agents, only now are we starting to appreciate the vast complexity of the cellular response to these drugs. The challenge ahead is to understand the reasons that underlie this complexity, and to use that knowledge as a means to improve the design and use of such therapies.

K.E.G. was funded by a studentship from the Biotechnology and Biological Sciences Research Council; S.S.T. is a Cancer Research UK Senior Fellow.

References

Allan, L. A. and Clarke, P. R. (2007). Phosphorylation of caspase-9 by CDK1/cyclin B1 protects mitotic cells against apoptosis. *Mol. Cell* **26**, 301-310.

Bergnes, G., Brejc, K. and Belmont, L. (2005). Mitotic kinesins: prospects for antimitotic drug discovery. *Curr. Top. Med. Chem.* **5**, 127-145.

Brinkley, B. R. (2001). Managing the centrosome numbers game: from chaos to stability in cancer cell division. *Trends Cell Biol.* **11**, 18-21.

Brito, D. A. and Rieder, C. L. (2006). Mitotic checkpoint slippage in humans occurs via cyclin B destruction in the presence of an active checkpoint. *Curr. Biol.* **16**, 1194-1200.

Brito, D. A. and Rieder, C. L. (2008). The ability to survive mitosis in the presence of microtubule poisons differs significantly between human nontransformed (RPE-1) and cancer (U2OS, HeLa) cells. *Cell Motil. Cytoskeleton* (in press). doi:10.1002/cm.20316.

Brito, D. A., Yang, Z. and Rieder, C. L. (2008). Microtubules do not promote mitotic slippage when the spindle assembly checkpoint cannot be satisfied. *J. Cell Biol.* **182**, 623-629.

Chen, J. G. and Horwitz, S. B. (2002). Differential mitotic responses to microtubule-stabilizing and -destabilizing drugs. *Cancer Res.* **62**, 1935-1938.

Gascoigne, K. E. and Taylor, S. S. (2008). Cancer cells display profound intra- and interline variation following prolonged exposure to antimitotic drugs. *Cancer Cell* **14**, 111-122.

Holland, A. J. and Cleveland, D. W. (2008). Beyond genetics: surprising determinants of cell fate in antitumor drugs. *Cancer Cell* **14**, 103-105.

Jordan, M. A. and Wilson, L. (1999). The use and action of drugs in analyzing mitosis. *Methods Cell Biol.* **61**, 267-295.

Jordan, M. A. and Wilson, L. (2004). Microtubules as a target for anticancer drugs. *Nat. Rev. Cancer* **4**, 253-265.

Jordan, M. A., Thrower, D. and Wilson, L. (1991). Mechanism of inhibition of cell proliferation by Vinca alkaloids. *Cancer Res.* **51**, 2212-2222.

Jordan, M. A., Toso, R. J., Thrower, D. and Wilson, L. (1993). Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. *Proc. Natl. Acad. Sci. USA* **90**, 9552-9556.

Jordan, M. A., Wendell, K., Gardiner, S., Derry, W. B., Copp, H. and Wilson, L. (1996). Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. *Cancer Res.* **56**, 816-825.

Karlsson, M. O., Molnar, V., Freijs, A., Nygren, P., Bergh, J. and Larsson, R. (1999). Pharmacokinetic models for the saturable distribution of paclitaxel. *Drug Metab. Dispos.* **27**, 1220-1223.

Keen, N. and Taylor, S. (2004). Aurora-kinase inhibitors as anticancer agents. *Nat. Rev. Cancer* **4**, 927-936.

Keen, N. and Taylor, S. (2009). Mitotic drivers-inhibitors of the Aurora B Kinase. *Cancer Metastasis Rev.* **28**, 185-195.

Kelling, J., Sullivan, K., Wilson, L. and Jordan, M. A. (2003). Suppression of centromere dynamics by Taxol in living osteosarcoma cells. *Cancer Res.* **63**, 2794-2801.

Klein, E., Debonis, S., Thiede, B., Skoufias, D. A., Kozielski, F. and Lebeau, L. (2007). New chemical tools for investigating human mitotic kinesin Eg5. *Bioorg. Med. Chem.* **15**, 6474-6488.

Knox, J. J., Gill, S., Synold, T. W., Biagi, J. J., Major, P., Feld, R., Cripps, C., Wainman, N., Eisenhauer, E. and Seymour, L. (2008). A phase II and pharmacokinetic study of SB-715992, in patients with metastatic hepatocellular carcinoma: a study of the National Cancer Institute of Canada Clinical Trials Group (NCIC CTG IND.168). *Invest. New Drugs* **26**, 265-272.

Kwon, M., Godinho, S. A., Chandhok, N. S., Ganem, N. J., Azioune, A., Thery, M. and Pellman, D. (2008). Mechanisms to suppress multipolar divisions in cancer cells with extra centrosomes. *Genes Dev.* **22**, 2189-2203.

Lanni, J. S. and Jacks, T. (1998). Characterization of the p53-dependent postmitotic checkpoint following spindle disruption. *Mol. Cell. Biol.* **18**, 1055-1064.

Lee, C. W., Belanger, K., Rao, S. C., Petrella, T. M., Tozer, R. G., Wood, L., Savage, K. J., Eisenhauer, E. A., Synold, T. W., Wainman, N. et al. (2008). A phase II study of ispinesib (SB-715992) in patients with metastatic or recurrent malignant melanoma: a National Cancer Institute of Canada Clinical Trials Group trial. *Invest. New Drugs* **26**, 249-255.

McGrogan, B. T., Gilmartin, B., Carney, D. N. and McCann, A. (2008). Taxanes, microtubules and chemoresistant breast cancer. *Biochim. Biophys. Acta* **1785**, 96-132.

Milross, C. G., Mason, K. A., Hunter, N. R., Chung, W. K., Peters, L. J. and Milas, L. (1996). Relationship of mitotic arrest and apoptosis to antitumor effect of paclitaxel. *J. Natl. Cancer Inst.* **88**, 1308-1314.

Musacchio, A. and Salmon, E. D. (2007). The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* **8**, 379-393.
Niikura, Y., Dixit, A., Scott, R., Perkins, G. and Kitagawa, K. (2007). BUB1 mediation of caspase-independent mitotic death determines cell fate. *J. Cell Biol.* **178**, 283-296.
Nogales, E. (2000). Structural insights into microtubule function. *Annu. Rev. Biochem.* **69**, 277-302.
Orth, J. D., Tang, Y., Shi, J., Loy, C. T., Amendt, C., Wilm, C., Zenke, F. T. and Mitchison, T. J. (2008). Quantitative live imaging of cancer and normal cells treated with Kinesin-5 inhibitors indicates significant differences in phenotypic responses and cell fate. *Mol. Cancer Ther.* **7**, 3480-3489.
Panvichian, R., Orth, K., Day, M. L., Day, K. C., Pilat, M. J. and Pienta, K. J. (1998). Paclitaxel-associated multimininucleation is permitted by the inhibition of caspase activation: a potential early step in drug resistance. *Cancer Res.* **58**, 4667-4672.
Rai, S. S. and Wolff, J. (1996). Localization of the vinblastine-binding site on beta-tubulin. *J. Biol. Chem.* **271**, 14707-14711.
Rieder, C. L. and Maiato, H. (2004). Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint. *Dev. Cell* **7**, 637-651.
Rowinsky, E. K. (1997). The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu. Rev. Med.* **48**, 353-374.
Rowinsky, E. K., Eisenhauer, E. A., Chaudhry, V., Arbuck, S. G. and Donehower, R. C. (1993). Clinical toxicities encountered with paclitaxel (Taxol). *Semin. Oncol.* **20**, 1-15.
Schiff, P. B., Fant, J. and Horwitz, S. B. (1979). Promotion of microtubule assembly *in vitro* by taxol. *Nature* **277**, 665-667.
Schimming, R., Mason, K. A., Hunter, N., Weil, M., Kishi, K. and Milas, L. (1999). Lack of correlation between mitotic arrest or apoptosis and antitumor effect of docetaxel. *Cancer Chemother. Pharmacol.* **43**, 165-172.
Schwartz, E. L. (2009). Antivascular actions of microtubule-binding drugs. *Clin. Cancer Res.* **15**, 2594-2601.
Shi, J., Orth, J. D. and Mitchison, T. (2008). Cell type variation in responses to antimitotic drugs that target microtubules and kinesin-5. *Cancer Res.* **68**, 3269-3276.
Strebhardt, K. and Ullrich, A. (2006). Targeting polo-like kinase 1 for cancer therapy. *Nat. Rev. Cancer* **6**, 321-330.
Symmans, W. F., Volm, M. D., Shapiro, R. L., Perkins, A. B., Kim, A. Y., Demaria, S., Yee, H. T., McMullen, H., Oratz, R., Klein, P. et al. (2000). Paclitaxel-induced apoptosis and mitotic arrest assessed by serial fine-needle aspiration: implications for early prediction of breast cancer response to neoadjuvant treatment. *Clin. Cancer Res.* **6**, 4610-4617.
Tang, P. A., Siu, L. L., Chen, E. X., Hotte, S. J., Chia, S., Schwarz, J. K., Pond, G. R., Johnson, C., Colevas, A. D., Synold, T. W. et al. (2008). Phase II study of ispinesib in recurrent or metastatic squamous cell carcinoma of the head and neck. *Invest. New Drugs* **26**, 257-264.
Tao, W., South, V. J., Zhang, Y., Davide, J. P., Farrell, L., Kohl, N. E., Sepp-Lorenzino, L. and Lobell, R. B. (2005). Induction of apoptosis by an inhibitor of the mitotic kinesin KSP requires both activation of the spindle assembly checkpoint and mitotic slippage. *Cancer Cell* **8**, 49-59.
Weaver, B. A. and Cleveland, D. W. (2005). Decoding the links between mitosis, cancer, and chemotherapy: the mitotic checkpoint, adaptation, and cell death. *Cancer Cell* **8**, 7-12.